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PERSPECTIVES IN RENAL MEDICINE

Difficulties in understanding human "acute tubular necrosis": Limited data and flawed animal models

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Difficulties in understanding human "acute tubular necrosis": Limited data and flawed animal models. This review summarizes the current understanding of the renal biopsy in "acute tubular necrosis" and the attempts to mimic this phenomenon in animal models. Paradoxically, only very limited necrosis is present in the biopsy of patients with this condition and differences in biopsies of patients with sustained and recovering renal failure cannot be clearly defined. The small amount of material examined, the variation in timing of the biopsy, the ability of the nephron to recover from sublethal injury, and the complexity of the clinical situation compound the difficulties in understanding this condition. Morphological findings in the animal studies are not equivalent to those in the human biopsy of "acute tubular necrosis," because they either have too much proximal tubular necrosis (ischemia-reflow model) or show severe injury to distal nephron segments (distal nephron model), the degree of which has not been clearly documented, as yet, in human material. The direct relevance of animal models in part may be tested by new noninvasive methods that define and quantify excreted proteins that reflect nephron injury or measure the status of renal oxygenation by radiological imaging techniques. Finally, it may be time to re-examine the morphology of "acute tubular necrosis," utilizing new techniques that illustrate induction of heat shock proteins, sublethal and apoptotic cellular injury, and alteration of gene expression.

Currently it is generally understood that the term "acute tubular necrosis"(ATN) does not accurately reflect the morphological changes in this condition [1]. In essence, ATN is the situation in which there is adequate renal perfusion such that there is sufficient blood flow to largely maintain tubular integrity, but not to sustain glomerular filtration. Indeed, in 1957 when the first renal biopsies were done by Brun and Munk in patients with ATN [2], they were struck by the fact that there was limited parenchymal compromise in spite of severe organ failure. These observations and their study were the beginning of a new approach to ATN. Their findings were supported by a concurrent autopsy study that found no distinctive lesion of ATN [3]. Years later, Solez and Finckh re-examined these data and did find limited, but significant tubular alterations that characterized patients with ATN [4]. These changes largely focused on distal tubular necrosis and regeneration with, interestingly, an inverse correlation between distal tubular necrosis and urine volume. Although inflammation per se is not considered part of ATN histology, in autopsy studies, the occurrence of vasa recta nucleated cells (possibly hematopoiesis) has been regarded by some investigators as a characteristic feature [5]. One light microscopic study disclosed two lesions significantly less severe in recovering ATN versus sustained ATN: necrosis of individual tubular cells and loss of PAS(+) brush border [6].

Renal biopsy studies published after those initial observations largely reinforced these renal biopsy and autopsy studies [1]. Further morphological advances involved ultrastructural analysis and were largely based on 25 biopsies [1, 7–9]. Eleven biopsies were from patients with established acute renal failure (ARF), ages varying from 24 to 71 years and obtained 2 to 25 days after onset of ARF; 14 biopsies were from patients in the recovery phase, ages varying from 17 to 64 years and obtained 2 to 30 days after onset of ARF. The etiologies of the ARF in these patients were shock following surgical operations, trauma, or postpartum hemorrhage as well as sepsis. In half of the patients, poisoning or nephrotoxicity was considered to have contributed to the ARF. The material examined by ultrastructure was limited, averaging 14 tubular profiles per patient (including both cortical and medullary tubules). Amazingly, despite this heterogeneity and sampling limitations, some conclusions could be drawn. In the cortex (principally convoluted tubules), single cell necrosis/desquamation with defects was found in both proximal tubules (0.8 ± 0.4%) and distal tubules (5.2 ± 2.9%). In the medulla, such changes were seen in S3 (3.7 ± 1.5%), medullary thick ascending limbs (10.7 ±

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3.2%), and collecting ducts (9.3 ± 5%). This distal tubular "necrosis" was significantly greater in sustained versus recovering renal failure [7]. There was cellular "simplification" of both proximal and distal tubules (loss of brush border and basolateral membranes). Thus, the major changes—at least in terms of cell necrosis/desquamation—appeared to be in the distal nephron, and the ratio of distal/proximal cell loss in the cortex was 6.5/1 and in the medulla 3/1. Immunohistochemical studies of human biopsies of ATN also point to distal nephron injury (diminished number of tubules staining for Tamm-Horsfall protein) as compared with normal human kidneys [10]. Thus, both proximal and distal nephron elements are abnormal in ATN, but the most severe injury appears in the distal nephron.

The appearance of ATN in the renal transplant is very similar to that of nontransplant ATN, that is, limited parenchymal injury and manifest renal failure [11]. The advantages of studying ATN in this setting are obvious. The kidney is relatively accessible to biopsy. Abundant patient material is available, and the clinical situation is relatively homogeneous. The finding in biopsies of transplant ATN [11], by light microscopy, is limited necrosis (more than nontransplant ATN), but the necrotic changes are significantly higher in ATN versus groups with stable renal function. On the other hand, fine structural studies show no clear distinction between transplant ATN and groups with stable function [11, 12]. In support of these observations, immunohistochemical studies using Na^+, K^+-ATPase could not distinguish between sustained and recovering human ATN [12]. A systematic morphological examination of the renal medulla in human transplant ATN has not been undertaken. Taken together, these results from biopsy studies of transplant ATN emphasize the disparity between the clinical findings of renal failure and the limited morphological changes. Furthermore, in this more homogeneous population, biopsies from sustained and recovering renal failure do not show clear differences.

In an attempt to understand the changes in human ATN, multiple animal models have been devised, and three such models particularly deserve mention. In the warm ischemia-reflow model, the renal artery is completely obstructed. The injury of ischemia-reflow depends on the time of obstruction, with more of the nephron recruited as this time increases [13]. During a recent three-year period, *Kidney International* published 17 studies in which the time of obstruction varied from 20 to 75 minutes, one third being one hour or more [14–30]. With short-term ischemia reflow, there is a high degree of variability of both extent and location of tubular injury. Maintenance of normal body temperature (true warm ischemia) is a critical factor in such experiments, that is, failure to maintain normal body temperature will result in markedly diminished injury [31]. Warm ischemia-reflow results in extensive necrosis destroying the proximal tubules of the outer stripe of the outer medulla, and with prolonged obstruction, the proximal convoluted tubules become necrotic as well [13]. Distal nephron involvement in this model is minimal, unless measures are taken that further reduce the medullary oxygen availability [32].

On the other hand, a few experimental studies describe the histology and physiology of the recovering kidney after cold ischemia-reflow [33–35]. Harvig et al examined transplanted rat kidneys after periods of 2, 12, and 16 hours of cold ischemia [33, 34]. They reported that "24 hours after transplantation of kidneys subjected to 12 and 16 hours of cold ischemia, small discrete areas of necrosis were seen in the proximal tubules, mainly in the pars recta in the outer stripe all the outer medulla . . . extensive necrotic areas comprising loops of Henle and collecting ducts were seen in the inner stripe of the outer zone of the medulla and to some extent in the inner zone of the medulla." The authors concluded that the functional impairment in these groups related to the widespread necrotic areas in the inner stripe and inner zone of the renal medulla. An analysis of the intrarenal hemodynamics in these kidneys (using Microfil and microsphere injection) showed a profound reduction of blood flow in the deep cortex and juxtamedullary glomeruli [35]. This kind of diminished medullary blood flow with continued glomerular filtration replicates the circumstances of a distal nephron model, as described next.

A third model was developed on the basis of studies with the isolated perfused kidney [36–38]. In these studies, because of energy requirements for solute transport, the medullary thick ascending limb proved extremely vulnerable to reduced oxygen availability. Under such conditions, both in vitro and vivo severe injury occurred within minutes [36, 39]. In vivo experiments were devised that minimized medullary oxygen availability with relative preservation of glomerular filtration [40, 41]. These studies resulted in several different models of ARF, culminating in the 1994 protocol, which included nitric oxide and prostaglandin inhibition in concert with radiocontrast administration [42]. When all three "insults" were employed, there was extensive medullary thick ascending limb damage that correlated with the degree of renal failure. For instance, when only nitric oxide and prostaglandin inhibition were used, medullary thick ascending limb injury was present but limited, and this injury did not correlate with the degree of renal failure. This lack of correlation probably related to the capacity of the medullary thick ascending limb to recover from moderate injury [43] or from other confounding factors such as activation of tubuloglomerular feedback.

How do these studies help us understand human ARF? Any conclusions from these experiments must be tempered by the morphological differences between the hu-

man kidney and the kidney of the rat, the animal utilized in almost all experimental studies. The latter has a well-developed medullary outer stripe, which in the human has a relatively limited representation. Furthermore, the vascular bundle of the inner stripe in the rat is complex (with inclusion of the thin descending limbs of the short loops of Henle). In humans, this structure is simple (without inclusion) [44]. On the other hand, despite these differences, a habitually low medullary pO_2 is a constant finding in all mammalian species including humans [45, 46].

The pathological changes in warm ischemia-reflow in rats are those of extensive tubular destruction and are not characteristic of human ATN. Furthermore, it is quite clear that therapeutic strategies effective in the rat to diminish the renal failure of warm ischemia-reflow have not, as yet, been successful in human ATN (abstract; Ramaswamy et al, *J Am Soc Nephrol* 11:595A, 2000) [47–49]. On the other hand, it is interesting that in the experiments of cold ischemia-reflow/transplant in the rat, distal nephron injury predominates; further studies using this model are indicated. As noted earlier, medullary changes in human renal transplant ATN have not been studied. The model of ATN in rats employing distal injury does utilize agents that cause human ATN [42], but the blatant morphological changes that characterize this model are present to a much more limited degree in human material. The paucity of such findings in the human patients who have been studied may relate to reversibility of distal nephron injury, the limited material available, and, perhaps, as well, the inattention to medullary injury by most pathologists.

Interestingly, renal failure in the distal nephron model is not improved by insulin-like growth factor-1 (IGF-1), as is the renal failure induced by warm ischemia-reflow [47, 50]. As the distal nephron model predicts in a pilot study, prostaglandin E_1 seems to be successful in prevention of renal dysfunction caused by radiocontrast media in high-risk patients [51].

It is important to realize that all of these models have engendered experimental studies that expand the understanding of renal injury. For instance, apoptotic cell death is a feature of both the ischemia-reflow [52] and distal nephron model [53], and is a documented event in the transplant biopsy as well [54]. However, the involved nephron segment in the latter is usually not clearly delineated [54]. Experiments in the ischemia-reflow model have defined the nature of sublethal proximal tubular injury documenting disruption of the actin cytoskeleton [55] and loss of the normal polarity of Na^+, K^+-ATPase [56]. The diminishment of tight junction integrity [57] and cell–matrix adhesion [58] in this situation provides the basis for understanding the tubular loss/detachment phenomena seen in human material. In this model of ischemia-reflow, heat shock proteins are induced and may be both cellular [59] and organ function protective [60], although the latter is controversial [19]. The intact distal nephron (medullary thick ascending limb; mTAL) responds with alterations of gene expression similar to those that are induced by growth factors in cell cultures (expression of egr-1, c-fos, KC, and JE) [61, 62] and, in addition, up-regulation of anti-apoptotic Bcl-2 genes [63]. The consequent growth factors and cytokines released by mTAL cells may aid in the recovery process. Of course, this contrasts sharply with the distal nephron model in which the focus of injury is the mTAL rather than the proximal tubule. Changes in gene expression and induction of heat proteins in this model are not defined yet.

The direct relevance of animal models to human disease in part may be tested by new noninvasive methods that define and quantify excreted proteins that reflect nephron injury [kidney injury molecule-1 (KIM-1) is a new biomarker for human renal proximal tubule injury; abstract; Han et al, *J Am Soc Nephrol* 11:129A, 2000] [64] or measure the status of renal oxygenation by radiological imaging techniques [46]. Finally, in the last few decades, extensive anatomical studies have shown the complex interstitial–tubulovascular relationships in the kidney that determine oxygen availability and, therefore, cellular injury [44, 65]. These relationships determine cellular survival, and recent in vitro experiments suggest that they may define the type of cell death (apoptotic or nonapoptotic) that occurs as well [66]. With this kind of new understanding and the ability to define critical changes in gene expression in histologically intact cells [67, 68], it may be time to re-examine the morphology of ATN.

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